

Primer Extension Coupled with Electrospray Product Ion Mass Spectrometry

A PCR product: 3' TTGATCAAGTATAGGGCCGATAA^GTCGGCATATGCGG...
 SNP primer 5' TAGTTCATATCCCGGCTATT

Step 1

50 nM PCR product, 3-4 μ M SNP primer
 1 μ M ddATP, 1 μ M ddCTP, 1 μ M ddGTP, 1 μ M ddTTP
 20 mM NH₄Ac buffer pH 8.7
 2 mM Mg(Ac)₂
 1 unit DNA polymerase
 15-20 thermal cycles

PCR product: 3' TTGATCAAGTATAGGGCCGATAA^GTCGGCATATGCGG...
 SNP primer 5' TAGTTCATATCCCGGCTATT^{Add}
 Cdd

Step 2

Reconstituted in 20 mM NH₄Ac buffer and
 pass sample through a metal chelating resin to
 remove magnesium from the solution.

Step 3 Analyze the sample solution using Selected Reaction Monitoring
 Electrospray Mass Spectrometry to detect the unreacted ddNTPs remaining
 in solution relative to a control sample that did not undergo primer extension.

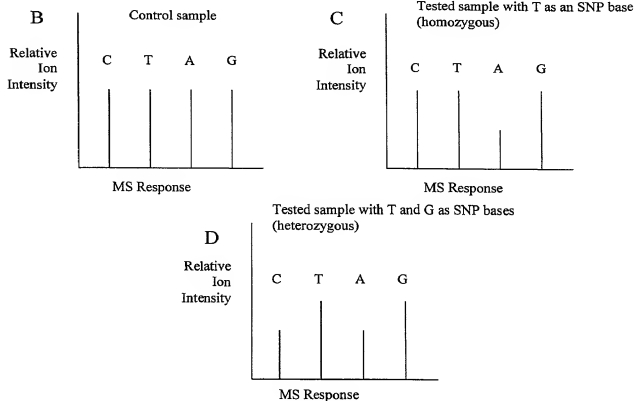


Figure 1

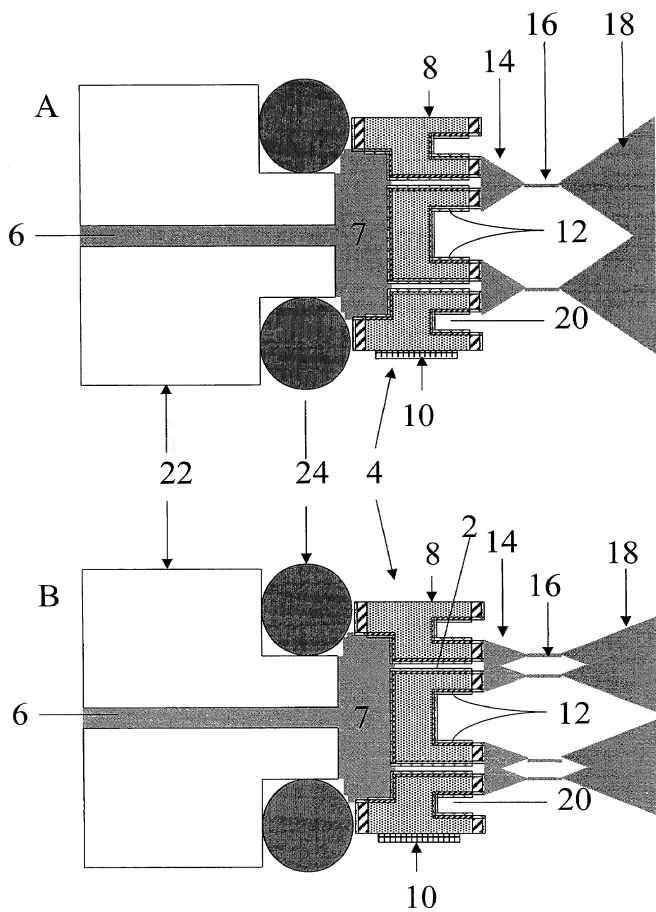


Figure 2

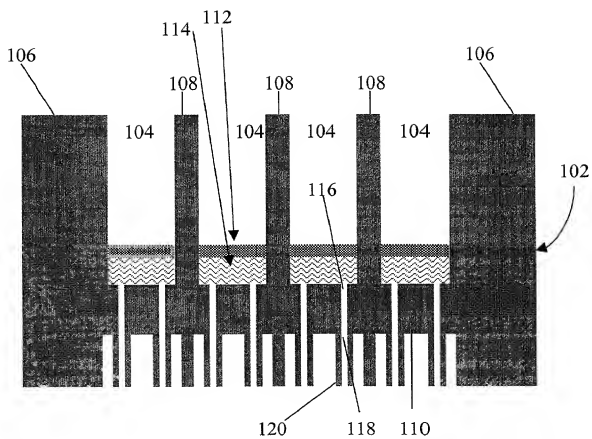


Figure 3A

Pressure to effect fluid transfer

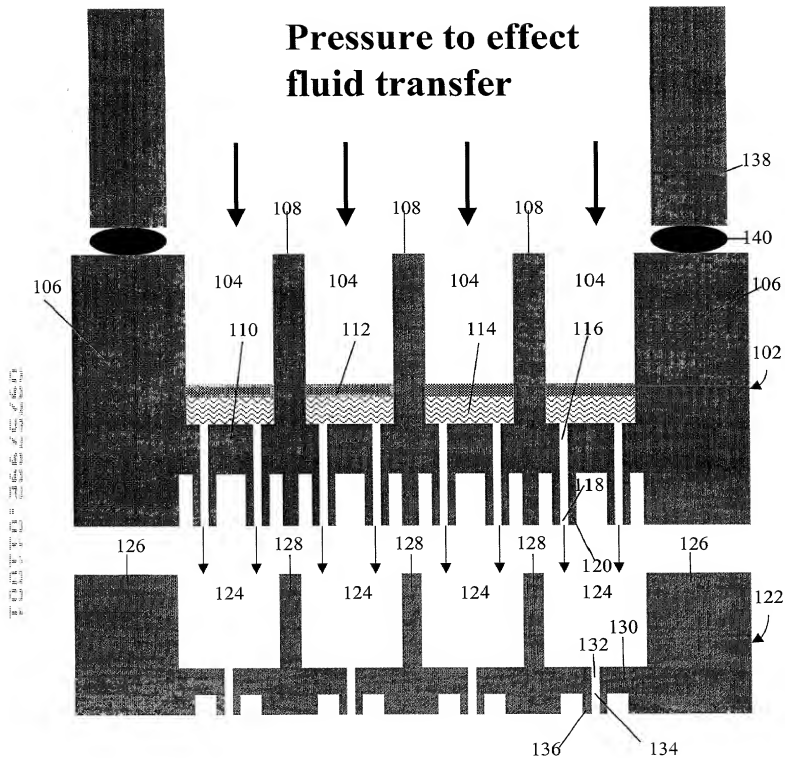


Figure 3B

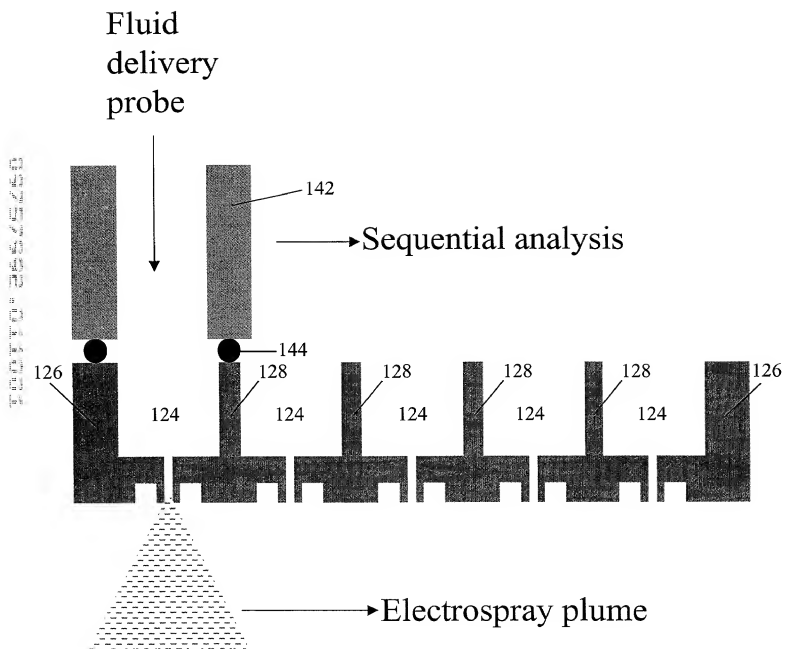


Figure 3C

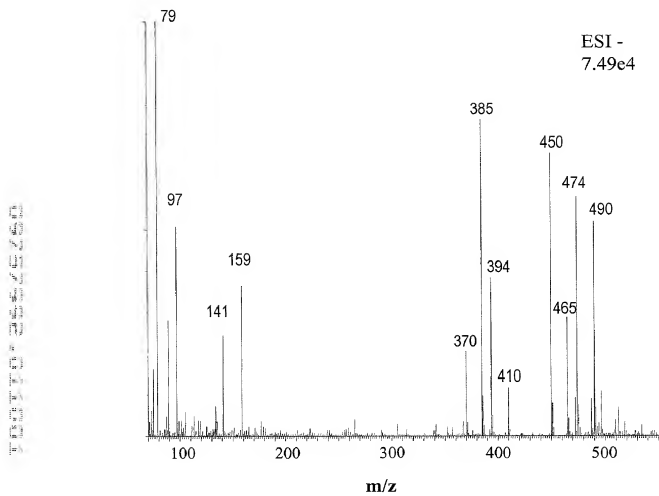


Figure 4

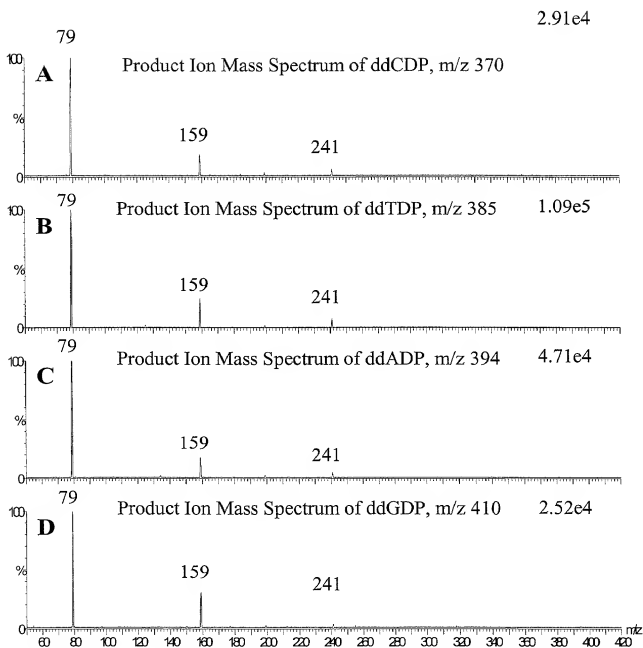


Figure 5

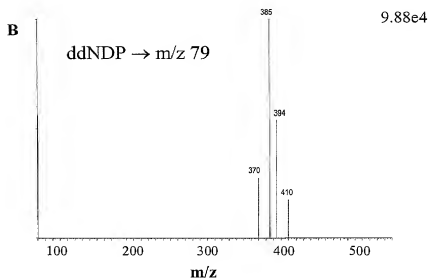
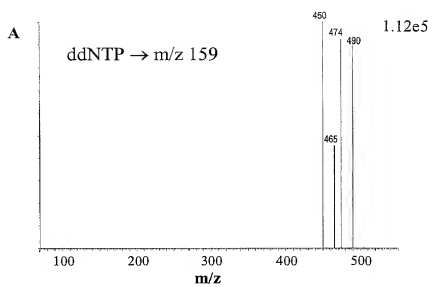


Figure 6

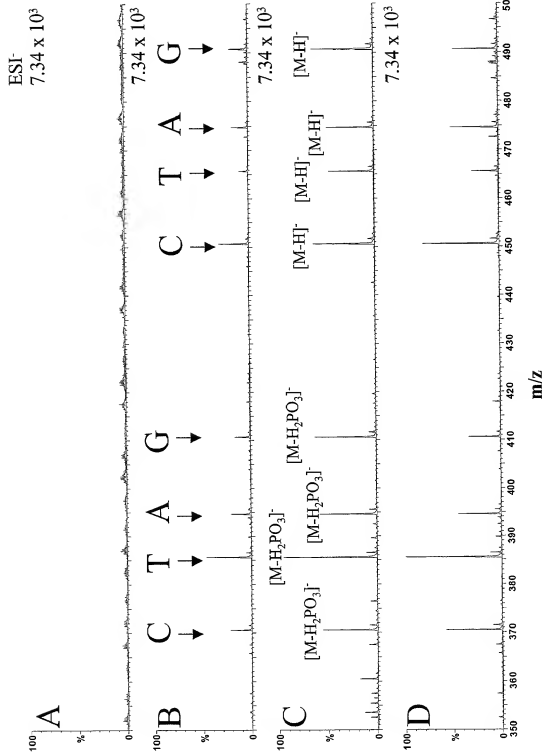


Figure 7

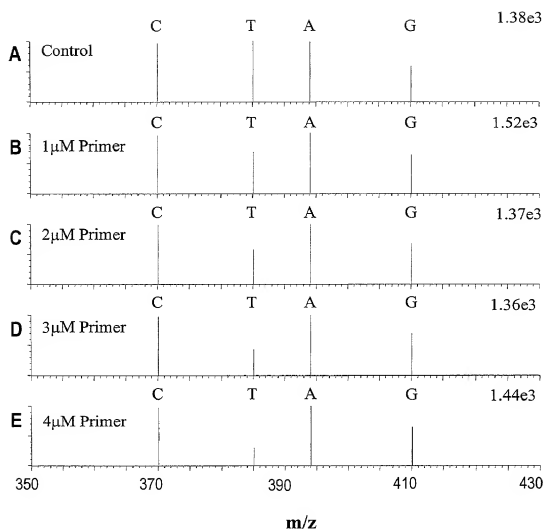


Figure 8

Schematic diagram of SNP genotyping by ESI/MS using synthetic single strand DNA as target templates in homogeneous reaction

Homogeneous templates:

Template A : 5' CCCCTGT**A**TCCTGTGTGAAATTGTTATCCGCTC 3' (SEQ. ID. No. 1)
 SNP primer: 3' AGGACACACTTTAACAATAGGCGA 5' (SEQ. ID. No. 5)
 Decreased ddNTPs: ddTTP (385.1>79)

Template C : 5' CCCCTGT**C**TCCTGTGTGAAATTGTTATCCGCTC 3' (SEQ. ID. No. 2)
 SNP primer: 3' AGGACACACTTTAACAATAGGCGA 5' (SEQ. ID. No. 5)
 Decreased ddNTPs: ddGTP (410.1>79)

Template G : 5' CCCCTGT**G**TCCTGTGTGAAATTGTTATCCGCTC 3' (SEQ. ID. No. 3)
 SNP primer: 3' AGGACACACTTTAACAATAGGCGA 5' (SEQ. ID. No. 5)
 Decreased ddNTPs: ddCTP (370.1>79)

Template T : 5' CCCCTGT**T**TCCTGTGTGAAATTGTTATCCGCTC 3' (SEQ. ID. No. 4)
 SNP primer: 3' AGGACACACTTTAACAATAGGCGA 5' (SEQ. ID. No. 5)
 Decreased ddNTPs: ddATP (394.1>79)

Figure 9

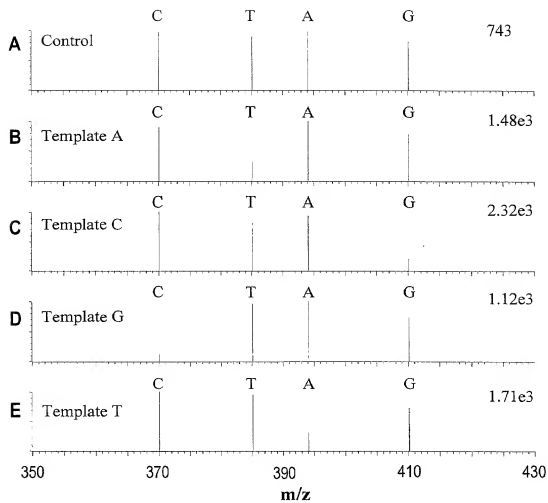


Figure 10

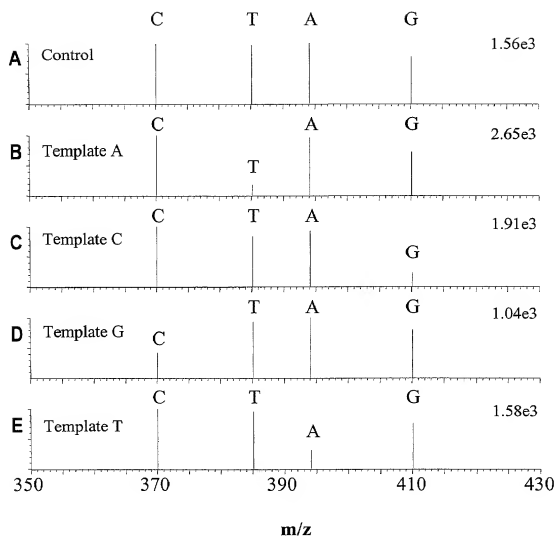


Figure 11

Schematic diagram of SNP genotyping by ESI/MS using synthetic single strand DNA as target templates in heterogeneous reaction

Heterogeneous templates (two templates mixture):

C (SEQ. ID. No. 2)

Template A+C: 5' CCCCTGT**A**TCCTGTGTGAAATTGTTATCCGCTC 3' (SEQ. ID. No. 1)

SNP primer: 3' AGGACACACTTTAACAATAGGCGA 5' (SEQ. ID. No. 5)

Decreased ddNTPs: ddTTP (385.1>79) and ddGTP (410.1>79)

G (SEQ. ID. No. 3)

Template A+G: 5' CCCCTGT**A**TCCTGTGTGAAATTGTTATCCGCTC 3' (SEQ. ID. No. 1)

SNP primer: 3' AGGACACACTTTAACAATAGGCGA 5' (SEQ. ID. No. 5)

Decreased ddNTPs: ddTTP (385.1>79) and ddCTP (370.1>79)

T (SEQ. ID. No. 4)

Template A+T: 5' CCCCTGT**A**TCCTGTGTGAAATTGTTATCCGCTC 3' (SEQ. ID. No. 1)

SNP primer: 3' AGGACACACTTTAACAATAGGCGA 5' (SEQ. ID. No. 5)

Decreased ddNTPs: ddTTP (385.1>79) and ddATP (394.1>79)

G (SEQ. ID. No. 3)

Template C+G: 5' CCCCTGT**C**TCCTGTGTGAAATTGTTATCCGCTC 3' (SEQ. ID. No. 2)

SNP primer: 3' AGGACACACTTTAACAATAGGCGA 5' (SEQ. ID. No. 5)

Decreased ddNTPs: ddGTP (410.1>79) and ddCTP (370.1>79)

T (SEQ. ID. No. 4)

Template C+T: 5' CCCCTGT**C**TCCTGTGTGAAATTGTTATCCGCTC 3' (SEQ. ID. No. 2)

SNP primer: 3' AGGACACACTTTAACAATAGGCGA 5' (SEQ. ID. No. 5)

Decreased ddNTPs: ddGTP (410.1>79) and ddATP (394.1>79)

T (SEQ. ID. No. 4)

Template G+T: 5' CCCCTGT**G**TCCTGTGTGAAATTGTTATCCGCTC 3' (SEQ. ID. No. 3)

SNP primer: 3' AGGACACACTTTAACAATAGGCGA 5' (SEQ. ID. No. 5)

Decreased ddNTPs: ddCTP (370.1>79) and ddATP (394.1>79)

Figure 12

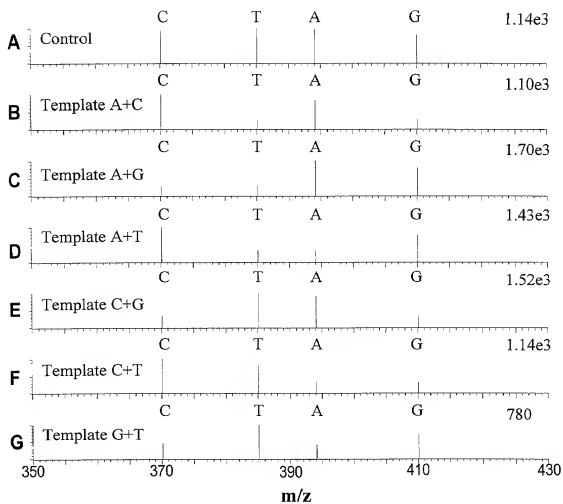


Figure 13

384 bp of partial PheA gene and its primers used for SNP assay:

384bp double-stranded target sequence: (SEQ. ID. No. 6)



Amplification primers:

W338Ipd 5'-CGGTAATCCAATTGAAGAGATGTTCT-3' (SEQ. ID. No. 7)

#1224 5'-CGCCAGGGTTTTCCAGTCACGA-3' (SEQ. ID. No. 8)

Polymorphism detection primers:

Polymorphism base

| | | |
|--|---|-------------------|
| W338Ipd 5'-CGGTAATCCAATTGAAGAGATGTTCT-3' | T | (SEQ. ID. No. 7) |
| C374Spu 5'-TCACTTGGGTAGGATCCCAATACCTTCATT-3' | C | (SEQ. ID. No. 9) |
| #1224 5'-CGCCAGGGTTTTCCAGTCACGA-3' | G | (SEQ. ID. No. 8) |
| C374Apd 5'-AGGTATTGGGCGCCTACCCAAAGTGAG-3' | T | (SEQ. ID. No. 10) |
| T366pd 5'-ACCCGTTCAATGAAGGTATTGGGC-3' | A | (SEQ. ID. No. 11) |
| V383pu 5'-AACAGGCACTACGTTCTCACTTGGGTA-3' | T | (SEQ. ID. No. 12) |

Figure 14

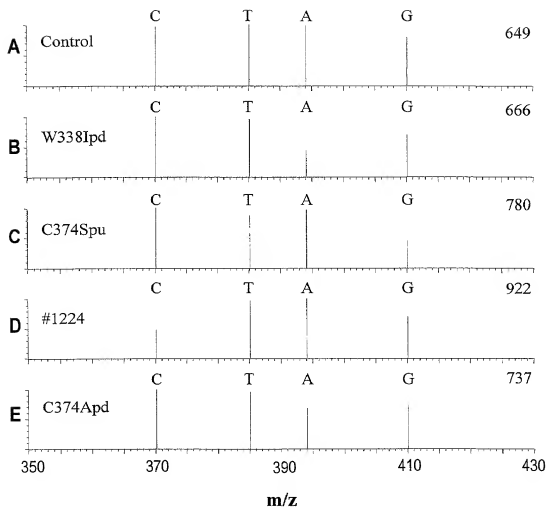


Figure 15

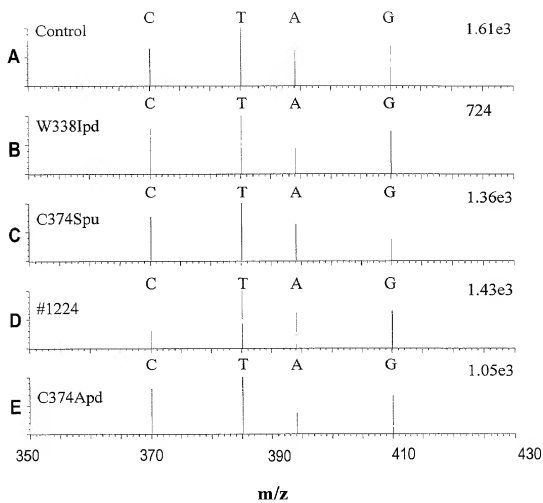
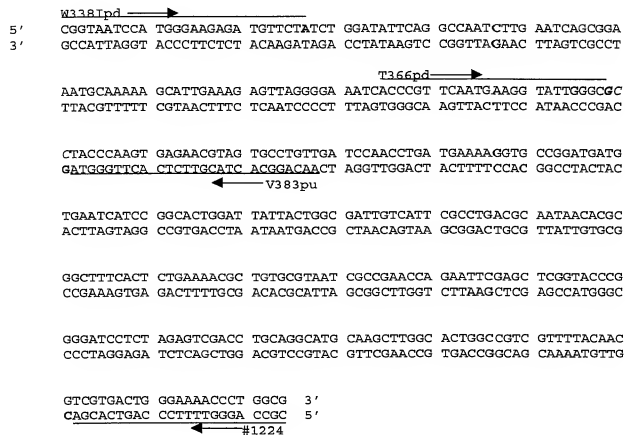


Figure 16

384 bp of partial *PheA*-C374A mutant gene and its primers used for SNP assay:

384bp double-stranded target sequence: (SEQ. ID. No. 13)



Amplification primers:

W3381pd 5'-CGGTAATCCAATTGAAGAGATGTTCT-3' (SEQ. ID. No. 7)
 #1224 5'-CGCCAGGGTTTTCCAGTCACGA-3' (SEQ. ID. No. 8)

Polymorphism detection primers:

Polymorphic base

| | | | |
|---------|-----------------------------------|---|-------------------|
| W3381pd | 5'-CGGTAATCCAATTGAAGAGATGTTCT-3' | A | (SEQ. ID. No. 7) |
| #1224 | 5'-CGCCAGGGTTTTCCAGTCACGA-3' | C | (SEQ. ID. No. 8) |
| T366pd | 5'-ACCCGTCAATGAAGGTATTGGGC-3' | C | (SEQ. ID. No. 11) |
| V383pu | 5'-AACAGGCACTACGTTCTCACTTGGGTA-3' | C | (SEQ. ID. No. 12) |

Figure 17

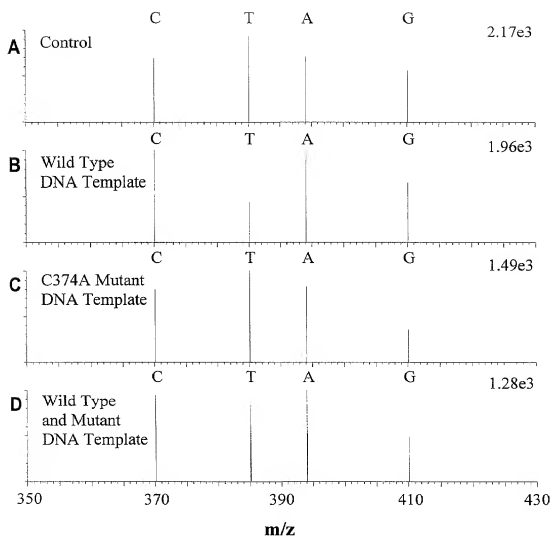


Figure 18

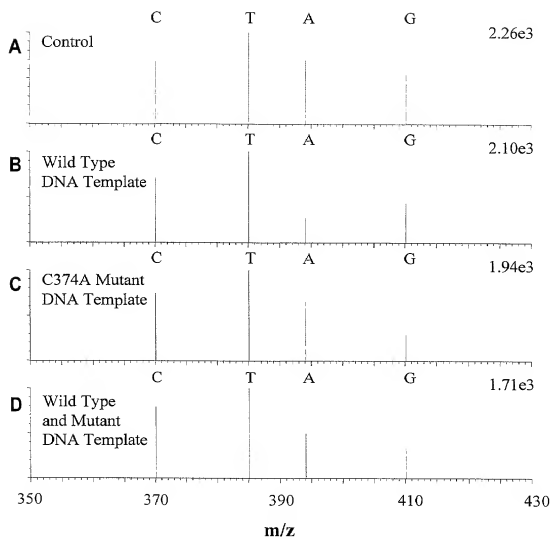


Figure 19

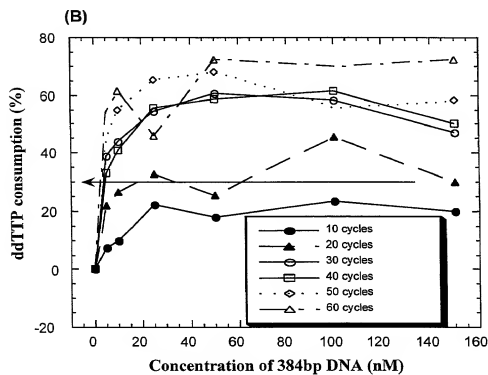
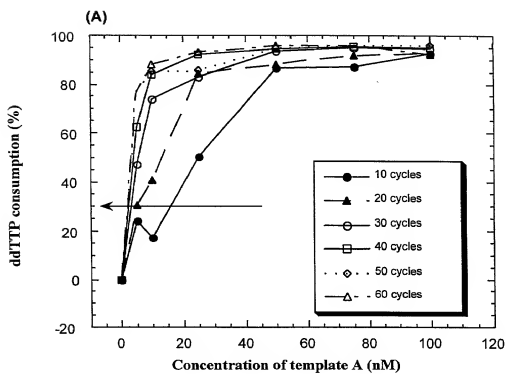


Figure 20